

GeneXP™ Human Angiogenesis Assay Kits

User Manual Version 1
Cat. No. HK 1401
Cat. No. HK 1402

Size: 1 KIT (40 rxns)

Stable for six months from the assay date. Recommended storage temperature -20°C. After thawing the mix can be refrozen or optionally stored at 4°C for three months.

1. INTRODUCTION

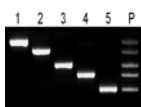
Angiogenesis is the physiological process involving the growth of new blood vessels from pre-existing vessels. These kits include the expression assay of 10 genes of the growth factors and receptors that play an important role in angiogenesis.

2. GeneXP™ ASSAY KIT DESCRIPTION

GeneXP™ Human Angiogenesis Assay Kits include all necessary PCR amplification reagents including the *Taq* DNA Polymerase. These kits are designed to direct the simultaneous amplification of specific regions of human cDNA. Kits offer Seegene's optimal primer/buffer system which will enhance amplification specificity. Figures show quality control PCR results obtained by following gene expression assay kits using positive control DNA.

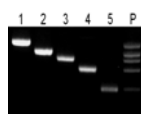
3. GeneXP™ ANGIOGENESIS PRIMER INFORMATION

A-HK 1401



Product Code	Lane	Gene	Size
Human Ango-1 Assay Kit	1	F2R	608bp
	2	ANGPT1	481bp
	3	FLT1	351bp
	4	TIE2	276bp
	5	FLT4	184bp
	P	MIX	

B-HK1402



Product Code	Lane	Gene	Size
Human Ango-2 Assay Kit	1	PAR2	577bp
	2	EDNRA	471bp
	3	PTAFR	409bp
	4	EDNRB	326bp
	5	GPR37	202bp
	P	MIX	

Quality control validation of GeneXP™ Human Angiogenesis Assay Kits with individual primer pair and multiplex primer sets, A: Human Ango-1, B: Human Ango-2, P: Positive Control DNA.

4. KEY FEATURES

4.1 High Specificity

GeneXP™ Human Angiogenesis Assay Kits detect all human resource previously used in other assays and generate only targeted bands in variable cancer cell line tests conducted by Seegene and collaborators. Non-specific bands, which were so often a problem of multiplex PCR were eliminated by adopting Seegene's DSO™ technology (patent pending) in primer design.

4.2 High Sensitivity

In general, the sensitivity of multiplex PCR is much lower than that of single PCR due to primer interference. The low sensitivity of multiplex PCR sometimes results in false negatives when using currently available multiplex gene expression profiling assay kits. However, our patented DSO™ technology greatly enhanced the sensitivity of multiplex PCR in GeneXP™ Human Angiogenesis Assay Kits.

4.3 Use of a Hot start *Taq* DNA Polymerase

GeneXP™ Human Angiogenesis Assay Kits use hot start *Taq* DNA polymerase, which, by inhibiting polymerase activity prior to PCR cycling, reduces non-specific amplification and increases target yield.

5. COMPONENTS

This kit is made up for the testing of 32 samples plus 8 controls (4 for positive controls and 4 for negative controls). We recommend that you use 1 negative and 1 positive control in every 8 samples.

Common components	Kit contains:	
		Volume
	2X Master mix	400 μ l
	Positive Control DNA	30 μ l
	User Manual	
HK 1401	5X Human ANGO-1 primer	160 μ l
HK 1402	5X Human ANGO-2 primer	160 μ l

6. PROCEDURE

6.1 SAMPLE PREPARATION (not included in this kit)

6.1.1 Total RNA isolation

The method of RNA isolation is critical for a successful expression profiling experiment. It is essential to purify RNA without any contaminating genomic DNA. Traces of genomic DNA in the RNA preparation may affect to final results.

6.1.2 Quantitation of RNA

1. Quantitate RNA samples by measuring the absorbance at 260 nm and 280 nm (A260 and A280) of an appropriate dilution (100 - 200 fold) in a spectrophotometer. For example, dilute 2 μ l sample with 198 μ l 1X TE Buffer, which is equivalent to a 1:100 dilution.

2. Calculate the concentration of the RNA sample.

Concentration of RNA (μ g/ml) = Absorbance at A260 x Dilution factor x 40 μ g/ml.

6.1.3 Checking the quality of RNA

1. A total RNA sample that is essentially free of proteins should have an A260/A280 ratio of 1.8 - 2.2.

2. To obtain accurate and reproducible results with the expression profiling assay, the use of high quality RNA is essential. The integrity of sample RNA and the amount of genomic DNA contamination should be assessed by agarose gel electrophoresis prior to the synthesis of cDNA. Run 1-2 μ g of total RNA either on a non-denaturing agarose/ethidium bromide gel to determine genomic DNA contamination or on a formaldehyde/agarose/ethidium bromide gel to examine RNA integrity. If excessive amounts of genomic DNA are present, it is necessary to treat the RNA sample with RNase-free DNase I.

Note: For details on running agarose gel electrophoresis, refer to Sambrook, J. et al.,(1989) Gel Electrophoresis of DNA. In Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press, N.Y., p. 6.1.

6.2 REVERSE TRANSCRIPTION (not included in this kit)

1. Add the following reagents to an RT tube on ice.

Components	Volume
Total RNA	500 ng
Oligo dT(10uM)	2 μ l
DEPC DW	? μ l
Total volume	10.5 μ l

Note: Mix the reagents by tapping or pipetting.

Note: Although we recommend 0.5 μ g of Total RNA, a wide range of total amounts (1 μ g ~ 3 μ g) give reproducibly good results.

2. Incubate the tube at 80°C for 3 min and chill the tube on ice for 2min and spin the tube briefly.

3. Add the following reagents to the tube from step 2.

Components	Volume
5X RT buffer	4 μ l
RNase inhibitor	20 units
MMLV RT	200 units
2.5mM dNTP	4 μ l
Total volume	20 μ l

4. Incubate the tube at 42°C for 90 min and heat the tube at 94°C for 2 min.

5. Chill the tube on ice for 2 min and spin the tube briefly.

6. Dilute (3.5volume=70 μ l) the cDNA by ddH₂O.

Note: Store all cDNA samples at -20°C until ready for use.

6.3 PCR PROTOCOL

6.3.1 Reaction Mixture Preparation

Set up PCR reactions with the test samples and PCR buffers provided in the PCR kit according to the table:

Components	Volume
cDNA	? μ l
5X Human ANGO-X primer mix	4 μ l
Sterilized water	? μ l
2X master mix	10 μ l
Total volume	20 μ l

Note: Depending on the samples, different amount (1-5 μ l) of diluted first strand cDNA can be used as templates for PCR.

6.3.2 PCR Cycle Parameter

Reaction profiles will need to be optimized according to the machine type and needs of user. Please take a note that temperature variations occur between different cyclers, therefore, the annealing temperature in the sample profile below is given as a range. It will be necessary to determine the optimal temperature for your individual cyler. An example of a time-temperature profile for the positive control PCR reaction optimized for

Perkin Elmer machine types GeneAmp PCR system 9700 is provided below:

Segment	No. of cycles	Temperature	Duration
1	1	94 °C	15 min
2	40	94 °C	0.5 min
		63 °C	1.5 min
		72 °C	1.5 min
3	1	72 °C	10 min
4		4 °C	soak

6.3.3 Agarose Gel Electrophoresis

Electrophoresis positive control (2 μl) and tested samples (4 μl) of the PCR products on a 2% agarose gel containing EtBr.

6.4 Positive Control DNA PCR

Positive control DNA contains a mixture of 5 genes control DNAs. For the control PCR, use 6 μl of the Positive Control DNA instead of the first-strand cDNA as template.

Components	Volume
Positive control DNA	6 μl
5X Human ANGO-X primer mix	4 μl
2X master mix	10 μl
Total volume	20 μl

Note: For PCR condition, please refer to section 6.3.2 PCR cycle parameter. Electrophoresis 2 μl of the control PCR products on a 2% agarose gel containing EtBr as a standard size marker and as a control for the PCR reaction. For the product sizes of apoptosis genes please refer to section 3 GeneXP™ Human Angiogenesis Primer Information.

6.5 Negative Control PCR

Use 6 μl of water instead of positive control DNA in the PCR mixture. No PCR band is expected in the negative control.

Note: Please be careful not to contaminate the human angiogenesis primer sets and 2X master mix with PCR products through pipetting. To prevent contamination of human angiogenesis primers sets and 2X master mix from PCR products, use of cotton tips is recommended. It is also recommended the same pipette not to use for the pipetting of PCR products and primers. Different pipettes for the exclusive pipetting of either one or the other should be reserved for this purpose.

7. TROUBLE SHOOTING

Problem	Cause	Solution
No Bands detected	The annealing temperature in cyclor is too high.	Decrease PCR annealing temperature 3-5°C.
Non-specific bands	The annealing temperature in the cyclor is too low.	Increase PCR annealing temperature 3-5°C.
No difference in gene expression among treatments	PCR amplification of this specific gene has passed the exponential phase.	Decrease PCR cycle number or decrease the input cDNA.
	Variation in sample preparation, RT reaction and amounts of input cDNA.	Run a parallel PCR with a house-keeping gene to eliminate variables.
	Genomic DNA contamination in total RNA	it is necessary to treat the RNA sample with RNase-free DNase I.
Amplicon bands in the negative control	Your cDNA or PCR reagents may be contaminated	To prevent contamination of angiogenesis primer sets and 2X master mix from PCR products, the use of cotton tips is recommended. It is also recommended that the same pipette not be used for the pipetting of PCR products and primers.

8. REFERENCES

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- Kerbel K. S. (2000) Tumor Angiogenesis: Past, Present and the Near Future. *Carcinogenesis* 21: 505-515.
- Carmeliet P, Jain R K. (2000) Angiogenesis in Cancer and Other Diseases. *Nature* 407: 249-257.
- Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, Pober JS, Wick TM, Konkle BA, Schwartz BS, Barnathan ES, McCrae KR, Hug BA, Schmidt AM, Stern DM. (1998) Endothelial Cells in Physiology and in the Pathophysiology of Vascular Disorders. *Blood* 91: 3527-61.
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- Vikkula M, Boon LM, Mulliken JB (2001) Molecular Genetics of Vascular

Malformations. Matrix Biol 20: 327-35.

9. RESEARCH USE ONLY

Since these products are intended for research purposes by qualified persons, the Environmental Protection Agency does not require us to supply Pre-manufacturing Notice.